PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

REC'D 16 AUG 2004
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(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 490224 NXK/jn	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/NZ2003/000224**	International Filing Date *(day/month/year)* 8 October 2003	Priority Date *(day/month/year)* 8 October 2002

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] A23L 3/015, 3/3571

Applicant

FONTERRA CO-OPERATIVE GROUP LIMITED et al

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 11 sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand 29 April 2004	Date of completion of the report 6 August 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No, (02) 6285 3929	Authorized Officer **Philippa Wyrdeman** Telephone No. (02) 6283 2632

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/NZ2003/000224

I. Basis of the report

1. With regard to the **elements** of the international application:*

- [ ] the international application as originally filed.
- [X] the description, pages **, 1, 3 and 7-29** as originally filed,
  pages , filed with the demand,
  pages **,2 and 4-6** received on **16/7/04** with the letter of **9/7/04**
- [X] the claims, pages , as originally filed,
  pages , as amended (together with any statement) under Article 19,
  pages , filed with the demand,
  pages **, 30-36** received on **16/7/04** with the letter of **9/7/04**
- [X] the drawings, pages **,1/4-4/4** as originally filed,
  pages , filed with the demand,
  pages , received on with the letter of
- [ ] the sequence listing part of the description:
  pages , as originally filed
  pages , filed with the demand
  pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).
- [ ] the language of publication of the international application (under Rule 48.3(b)).
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in written form.
- [ ] filed together with the international application in computer readable form.
- [ ] furnished subsequently to this Authority in written form.
- [ ] furnished subsequently to this Authority in computer readable form.
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages
   - [ ] the claims, Nos.
   - [ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-47	YES
	Claims		NO
Inventive step (IS)	Claims	1-47	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-47	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following citation from the search report are referred to in this report:

D1 = GB 2367997A. 24 April 2002.

D2 = Ulmer, H. M. et al. 2000. Applied and Environmental Microbiology. 66 (9) pp 3966-3973.

D3 = Calik, H. et al. 2002. Journal of food science Vol: 67, 4, pp 1506-1510.

The invention is directed to a method of treating food comprising at least one strain of a culture capable of surviving a pressure treatment of pre-determined pressure and pH, which prevents growth of spoilage microflora.

D1 is directed to a process of killing micro-organisms under super-atmospheric pressure comprising exposing the microbes to a peroxidase system and a super-atmospheric pressure between 100-1000 MPa. Although this document discloses the use of an UHP treatment between 100-1000MPa at 20-100 degrees Celsius it does not suggest the use of bacterial microbes capable of surviving said UHP.

D2 discloses the effect of high pressure processing on *Vibrio parahaemolyticus* through the use of hydrostatic pressures up to 1035 MPa. Although this document discloses the use of hydrostatic pressures up to 1035MPa it does not disclose the applicant's method.

D3 discloses the effects of high pressure on survival and metabolic activity of Lactobacillus plantarum TMW1.460 via the treatment of food with high pressures of 200-800 MPa. Although this document discloses the effect of a UHP treatment on a beer spoilage micro-organism it does not teach toward the applicants method.

As such D1-D3 do not suggest or teach toward the applicant's invention. Therefore the applicant's method is considered novel and inventive over the prior art and industrially applicable.

for delivery by ingestion. However, it is difficult to deliver such bacteria in sufficient numbers in a food that is subsequently heat-treated.

It is an object of the present invention to provide an improved or alternative method of treating a food product, and / or to go at least some way to overcoming the problems encountered with the prior art.

SUMMARY OF INVENTION

In one aspect the invention broadly comprises of a method of treating a food comprising the following steps:

- selecting a food comprising at least one strain of a culture, said strain capable of surviving a pressure treatment at a predetermined pressure and pH, and
- subjecting the food to a treatment pressure at or below the predetermined pressure, wherein the treatment pressure reduces, delays, prevents or eliminates growth of spoilage microflora;

wherein the treatment pressure is at least 350MPa.

Useful treatment pressures according to the invention may be selected from 350MPa, 360MPa, 370MPa, 380MPa, 390MPa, 400MPa, 410MPa, 420MPa, 430MPa, 440MPa 450MPa, 460MPa, 470MPa, 480MPa, 490MPa, 500MPa, 510MPa, 520MPa, 530MPa, 540MPa, 550MPa, 560MPa, 570MPa, 580MPa, 590MPa, 600MPa, 610MPa, 620MPa, 630MPa, 640MPa and 650MPa.

Preferably the food is subjected to a pressure of at least 400MPa.

It is envisaged that the invention may be performed at a pH level selected from the following: 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7,

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August 1997, *Streptococcus thermophilus* St10, *Streptococcus thermophilus* St49, *Lactobacillus helveticus* Lh1, *Lactobacillus helveticus* Lh5001, *Lactobacillus delbrukeii subsp bulgaricus* Lb1, Rhodia MY900 (commercially sold by Rhodia under the trade mark "MY900"), Rhodia MY105, Rhodia MYE95, Rhodia MYBio6, Rhodia TA060, Rhodia LH100, Chr. Hansen ABT4, Chr. Hansen YC-X11, Chr. Hansen ABT3, Danisco V1, Danisco Yo Mix VW, Danisco MSK Mix ABN1-45, *Bifidobacterium* lactis Bb12 (commercially sold by Nestle under the trade mark "Bb12"), *Bifidobacterium lactis* Wisby 420 (commercially sold by Wisby under the trade mark "420") and combinations thereof. The strains identified as St10, St49, Lh1, Lh5001 and Lb1 are commercially available from the Fonterra Research Centre Limited, Palmerston North, New Zealand.

In a second aspect, the invention broadly comprises of a method of treating a food, comprising the steps:

- selecting a food containing at least one strain of a culture, said strain being a probiotic strain capable of surviving a pressure treatment at a predetermined pressure and pH, and
- subjecting the food to a treatment pressure at or below the predetermined pressure, wherein the treatment pressure reduces, delays, prevents or eliminates growth of spoilage microfloral;

wherein the treatment pressure is at least 350MPa.

It is envisaged that the probiotic may be either used to ferment the food, or may be added to the food directly.

Probiotic strains used in the invention may be selected from strains of *Bifidobacterium*, preferably *Bifidobacterium lactis.*

Preferred probiotic strains used in the invention are selected from *Bifidobacterium lactis* HN019 AGAL deposit number NM 97/09513 dated 18 August 1997, and *Bifidobacterium* sold under the trade names Bb12 (Nestle) and Wisby 420.

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Other preferred probiotic strains used in the invention are selected from strains of *Lactobacillus*, preferably *Lactobacillus acidophilus*.

Most preferably a probiotic strain used in the invention is *Lactobacillus acidophilus* HN017 AGAL deposit number NM 97/09515 dated 18 August 1997.

Useful treatment pressures according to the invention may be selected from 350MPa, 360MPa, 370MPa, 380MPa, 390MPa, 400MPa, 410MPa, 420MPa, 430MPa, 440MPa 450MPa, 460MPa, 470MPa, 480MPa, 490MPa, 500MPa, 510MPa, 520MPa, 530MPa, 540MPa, 550MPa, 560MPa, 570MPa, 580MPa, 590MPa, 600MPa, 610MPa, 620MPa, 630MPa, 640MPa and 650MPa.

Preferably the pressure is at least 400MPa.

Alternatively the pressure is at least 500MPa.

It is envisaged that the invention may be performed at a pH level selected from the following: 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5 and 4.6.

In the preferred embodiment, the food is at a pH of between 3.0 and 4.6 when subjected to the treatment pressure.

Preferred conditions of temperature are as noted for the first aspect of the invention.

In a third aspect the invention broadly comprises of a method of treating a food comprising the following steps:

- selecting a food containing at least one strain of a protective culture, said strain capable of surviving a pressure treatment at a predetermined pressure and pH, and

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- subjecting the food to a treatment pressure at or below the predetermined pressure, wherein the treatment pressure reduces, delays, prevents or eliminates growth of spoilage microflora;

wherein the treatment pressure is at least 350MPa.

Preferably the protective culture is selected from those used in cultured dairy foods, fermented foods, cooked meats, vegetables, salads, cook-chilled foods, ready-to-eat foods. Such protective cultures include, but are not limited to, probiotics, bacteriocins and acid producing bacteria.

Preferred conditions of pH and temperature are as noted for the first aspect of the invention.

In a fourth aspect the invention consists in the use of at least one bacterial strain in a food to be subjected to a pressure treatment at a predetermined pressure of at least 350MPa such that undesired microflora are inactivated while the bacterial strain survives, said bacterial strain being selected from: *Lactobacillus acidophilus*, *Bifidobacterium lactis*, *Lactobacillus acidophilus* HN017 AGAL deposit number NM97/09515 dated 18 August 1997, *Bifidobacterium lactis* HN019 AGAL deposit number NM97/09513 dated 18 August 1997, *Streptococcus thermophilus* St10, *Streptococcus thermophilus* St49, *Lactobacillus helveticus* Lh1, *Lactobacillus helveticus* Lh5001, *Lactobacillus delbruekeii subsp bulgaricus* Lb1, Rhodia MY900, Rhodia MY105, Rhodia MYE95, Rhodia MYBio6, Rhodia TA060, Rhodia LH100, Chr. Hansen ABT4, Chr. Hansen YC-X11, Chr. Hansen ABT3, Danisco V1, Danisco Yo Mix VW, Danisco MSK Mix ABN1-45, and *Bifidobacterium* sold under the trade names Bb12 (Nestle) and Wisby 420 (Wisby).

According to the aspects of the invention, the foods may be subjected to the treatment pressure for between about 1 second and about 10 minutes. Preferred times may be selected from 1 second, 5 seconds, 10 seconds, 20 seconds, 30 seconds, 60 seconds, 90 seconds, 2 minutes, 3 minutes, 4 minutes, 5 minutes, 6 minutes, 7 minutes, 8 minutes, 9 minutes or 10 minutes.

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What we claim is:

1. A method of treating a food comprising the following steps:
   - selecting a food comprising at least one strain of a culture, said strain capable of surviving a pressure treatment at a predetermined pressure and pH, and
   - subjecting the food to a treatment pressure at or below the predetermined pressure, wherein the treatment pressure reduces, delays, prevents or eliminates growth of spoilage microflora;

   wherein the treatment pressure is at least 350MPa.

2. A method according to claim 1 wherein the treatment pressure is at least 400MPa.

3. A method according to any one of the preceding claims wherein the food is at a pH of between 3.0 and 8.0 when subjected to the treatment pressure.

4. A method according to claim 3 wherein the pH is between 3.6 and 4.8.

5. A method according to claim 4 wherein the pH is between 4.0 and 4.6.

6. A method according to any one of the preceding claims wherein the food is a cultured dairy product.

7. A method according to claim 6 wherein the cultured dairy product is yoghurt.

8. A method according to any one of claims 1 to 5 wherein the food is selected from a yoghurt drink, dairy dessert, cottage cheese, cream cheese and cultured beverages.

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9. A method according to any one of the preceding claims wherein the strain of culture is selected from:

- i) *Lactobacillus acidophilus*
- ii) *Bifidobacterium lactis*
- iii) *Streptococcus thermophilus;*
- iv) *Lactobacillus helveticus;*
- v) *Lactobacillus delbrukeii subsp bulgaricus*;

or any combination thereof.

10. A method of treating a food, comprising the steps:

- selecting a food comprising at least one strain of a culture, said strain being a probiotic strain capable of surviving a pressure treatment at a predetermined pressure and pH, and
- subjecting the food to a treatment pressure at or below the predetermined pressure, wherein the treatment pressure reduces, delays, prevents or eliminates growth of spoilage microflora;

wherein the treatment pressure is at least 350MPa.

11. A method according to claim 11 wherein the probiotic strain is *Bifidobacterium.*

12. A method according to claim 11 wherein the probiotic strain is *Bifidobacterium lactis.*

13. A method according to claim 12 wherein the probiotic strain is *Bifidobacterium lactis* HN019 AGAL deposit number NM 97/09513 dated 18 August 1997.

14. A method according to claim 10 wherein the probiotic strain is *Lactobacillus.*

15. A method according to claim 14 wherein the probiotic strain is *Lactobacillus acidophilus.*

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16. A method according to claim 15 wherein the probiotic is *Lactobacillus acidophilus* HN017 AGAL deposit number NM 97/09515 dated 18 August 1997.

17. A method according to any one of claims 10 to 16 wherein the treatment pressure is at least 400MPa.

18. A method according to claim 17 wherein the treatment pressure is at least 500MPa.

19. A method according to any one of claims 10 to 18 wherein the food is at a pH of between 3.0 and 4.6 when subjected to the treatment pressure.

20. A method according to any one of claims 10 to 19 wherein the food is selected from a yoghurt, a cultured dairy product, a beverage, a fruit juice or a vegetable juice.

21. A method of treating a food comprising the following steps:

- selecting a food comprising at least one strain of a protective culture, said strain capable of surviving a pressure treatment at a predetermined pressure and pH, and
- subjecting the food to a treatment pressure at or below the predetermined pressure, wherein the treatment pressure reduces, delays, prevents or eliminates growth of spoilage microflora;

wherein the treatment pressure is at least 350MPa.

22. The use of at least one bacterial strain in a food wherein said food is to be subjected to a treatment pressure of at least 350MPa wherein the treatment pressure reduces, delays, prevents or eliminates growth of spoilage microflora, and the bacterial strain survives, said bacterial strain being selected from:

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- i) *Lactobacillus acidophilus* HN017 AGAL deposit number NM97/09515 dated 18 August 1997;
- ii) *Bifidobacterium lactis* HN019 AGAL deposit number NM97/09513 dated 18 August 1997;
- iii) *Streptococcus thermophilus*;
- iv) *Lactobacillus helveticus*;
- v) *Lactobacillus delbruekeii subsp bulgaricus*;
- vi) *Lactobacillus acidophilus;*
- *vii) Bifidobacterium lactis;*

or any combination thereof.

23. A method of treating a food comprising the following steps:
- selecting a food comprising *Lactobacillus acidophilus* HN017 AGAL deposit number NM97/09515 dated 18 August 1997; and
- subjecting the food to a treatment pressure of between 350MPa and 600MPa, at a pH of between about 3.0 and about 8.0.

24. A method of treating a food comprising the following steps:
- selecting a food comprising *Bifidobacterium lactis* HN019 AGAL deposit number NM97/09513 dated 18 August 1997; and
- subjecting the food to a treatment pressure of between 350MPa and 600MPa, at a pH of between about 3.0 and about 8.0.

25. A method according to any one of the preceding claims wherein the food is subjected to the treatment pressure for less than 10 minutes.

26. A method according to claim 25 wherein the food is subjected to the treatment pressure for about 5 minutes.

27. A method according to claim 25 wherein the food is subjected to the treatment pressure less than 5 minutes.

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28. A method according to claim 27 wherein the food is subjected to the treatment pressure for about 1 minute.

29. A method according to claim 27 wherein the food is subjected to the treatment pressure for less than 1 minute.

30. A method according to claim 29 wherein the food is subjected to the treatment pressure for less than 30 seconds.

31. A method according to claim 30 wherein the food is subjected to the treatment pressure for less than 5 seconds.

32. A method according to claim 31 wherein the food is subjected to the treatment pressure for about 1 second.

33. A method according to any one of the preceding claims wherein the food is subjected to the treatment pressure at a temperature between about 0 degrees Celsius and 40 degrees Celsius.

34. A method according to claim 33 wherein the food is subjected to the treatment pressure at a temperature between about 0 degrees Celsius and 20 degree Celsius.

35. A food prepared by method according to any one of the preceding claims.

36. A food according to claim 35 wherein the food is selected from a yoghurt, a cultured dairy product, a beverage or a fruit or vegetable juice.

37. A cultured dairy product having a pH of at least 4.0 and a viable culture of at least one hundred thousand colony-forming units per gram following a pressure treatment of at least 400 MPa.

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38. A cultured dairy product with a pH of at least 4.0 having a viable culture of at least one hundred thousand colony-forming units per gram following a pressure treatment of at least 450 MPa.

39. A cultured dairy product with a pH of at least 4.0 having a viable culture of at least one hundred thousand colony-forming units per gram following a pressure treatment of at least 500 MPa.

40. A yoghurt or yoghurt drink with a pH of at least 4.0 having a viable culture of at least one hundred thousand colony-forming units per gram following a pressure treatment of at least 600MPa.

41. A food or beverage having a viable culture count of at least one hundred thousand colony-forming units per gram of at least one strain of a probiotic bacteria following a pressure treatment of at least 400 MPa for less than 10 mins.

42. A food or beverage having a viable culture count of at least one hundred thousand colony-forming units per gram of at least one strain of a probiotic bacteria following a pressure treatment of at least 450 MPa for less than 10 mins.

43. A method according to any one of claims 1 to 34 wherein the food has been packaged prior to being subjected to the treatment pressure.

44. Food made by the method according to any one of claims 1 to 34 wherein the spoilage organisms are inhibited for an extended period of time during storage, said extended period of time being longer than that achieved by an untreated food containing a strain of culture.

45. Food according to claim 44 wherein said storage is for at least 50 days at about 4 degrees Celsius.

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46. Food according to claim 44 wherein said storage is for at least 90 days at about 4 degrees Celsius.

47. Food according to claim 44 wherein said storage is for at least 15 days at 20 degrees Celsius.

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